

Kinetic vs. energetic discrimination in biological copying

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Abstract

We study stochastic copying schemes in which discrimination between a right and a wrong match is achieved via different kinetic barriers or different binding energy of the two matches. We demonstrate that, in single-step reactions, the two discrimination mechanisms are strictly alternative and can not be mixed to further reduce the error fraction. Close to the lowest error limit, kinetic discrimination results in a diverging copying velocity and dissipation per copied bit. On the opposite, energetic discrimination reaches its lowest error limit in an adiabatic regime where dissipation and velocity vanish. By analyzing experimentally measured kinetic rates of two DNA polymerases, T7 and Pol γ , we argue that one of them operates in the kinetic and the other in the energetic regime. Finally, we show how the two mechanisms can be combined in copying schemes implementing error correction through a proofreading pathway.

Living organisms need to process signals in a fast and reliable way. Copying information is a task of particular relevance, as it is required for the replication of the genetic code, the transcription of DNA into mRNA, and its translation into a protein. Reliability is fundamental, since errors can result in the costly (or harmful) production of a non-functional protein. Indeed, cells have developed mechanisms to reduce the copying error rate η to values as low as $\eta \sim 10^{-4}$ for protein transcription-translation [1] and $\eta \sim 10^{-10}$ for DNA replication [2]. Such mechanisms include multiple discrimination steps [1, 2] and pathways to undo wrong copies as in proofreading [2, 4, 5, 6] or backtracking [7].

Biological information is copied by thermodynamic machines that operate at a finite temperature. There is agreement that this fact alone implies a lower limit on the error rate. However, contrasting results have been obtained regarding the nature of this limit. In particular, it is not clear when it is reached in a slow and quasi-adiabatic regime, or in a fast and dissipative one. As clarified by

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Bennett [8], information can be copied adiabatically. Indeed, the copying scheme proposed in Hopfield's seminal proofreading paper [2] reaches its minimum error at zero velocity and zero dissipation [9]. In contrast, a copolymerization model proposed few years later by Bennett [1, 11, 12], achieves its minimum error in a highly dissipative regime, where velocity and dissipation diverge. Some of the biological literature has favoured that the minimum error is achieved in near-equilibrium conditions [9]. This view is however not unanimous [13]. Recent biophysical literature supports a dissipative minimum error limit [14, 11, 15, 12]. Similar disagreements are also present in models including proofreading. The proofreading model in [1] dissipates systematically less than the corresponding copying, while in other models [2, 4], at low errors, dissipation comes mainly from the proofreading step.

In this Letter, we show how these contrasting results can be rationalized noting that copying can be performed either discriminating through binding energies adiabatically, *energetic discrimination*, or discriminating through binding barriers dissipatively, *kinetic discrimination*. We begin by presenting a model for copying a single bit of information in the spirit of those proposed in [8, 16, 17, 18], see Fig. 1a. A bio-machine such as a polymerase binds and unbinds monomers of different species to a template, trying to match it. We then move to the case of copolymerization, Fig. 1b, where a polymerase assembles a polymer chain to match a template strand. Finally, we discuss two proofreading schemes, Figs. 1c and 1d, where the polymerase is assisted by an exonuclease that tends to remove wrong matches.

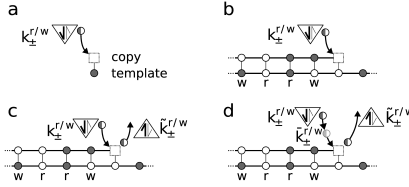


Fig. 1: a) Copying of one bit. The lower-vertex triangle represents a bio-machine, such as a polymerase, binding or unbinding right and wrong matches with rates $k_{\pm}^{r/w}$. b) Copolymerization. A template strand (bottom) is copied into a new strand (top). Right (r) and wrong (w) matches are added and removed with rates $k_{\pm}^{r/w}$. c) & d) Proofreading schemes. The polymerase is assisted by an exonuclease, represented by an upper-vertex triangle and characterized by $\tilde{k}_{\pm}^{r/w}$. In d), copies are made via an intermediate state characterised by $\bar{k}_{\pm}^{r/w}$.

Stochastic copying strategies of a single bit. The copying machine is described as a three-states system. Two are bound states in which the right (r) or wrong (w) molecule is attached to the machine. The third is a “blank” state (\emptyset), representing the state of the machine before a matching is done. To help physical intuition and following [1], we define the rates from the free energy

landscape in Fig. 2a. Right and wrong matching are characterized by a difference in barrier height δ , and in the energy of the final states γ . The energy ϵ is a chemical driving.

The four rates $k_{\pm}^{r/w}$ connecting the unbound state with the right and wrong states can be written (see Fig. 2a) as:

$$k_+^r = \omega e^{\epsilon+\delta} ; \quad k_-^r = \omega e^{\delta} ; \quad k_+^w = \omega e^{\epsilon} ; \quad k_-^w = \omega e^{\gamma}. \quad (1)$$

where ω is an overall rate scale. The master equation for the probabilities p_r and p_w of finding the system in the right or wrong state reads

$$\begin{aligned} \dot{p}_r &= (1 - p_r - p_w)k_+^r - k_-^r p_r \\ \dot{p}_w &= (1 - p_r - p_w)k_+^w - k_-^w p_w \end{aligned} \quad (2)$$

where p_\emptyset has been eliminated by normalization. We study the time-dependent

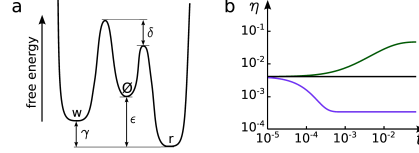


Fig. 2: a) Energy diagram for copying rates. The barrier height difference δ , energy difference between right and wrong states γ , and chemical driving ϵ are shown. b) Time-evolution of the error in the single bit copy for three parameter choices: $\gamma < \delta$ (green curve, $\gamma = 3$ and $\delta = 5.5$), $\gamma > \delta$ (blue curve, $\gamma = 8$ and $\delta = 5.5$), and $\gamma = \delta = 5.5$ (black curve). The other parameters are $\epsilon = 5$ and $\omega = 4$.

error rate $\eta(t) = p_r(t)/[p_r(t) + p_w(t)]$ for the system prepared in the unbound state, $p_r(t=0) = p_w(t=0) = 0$. At short times, $t \ll \omega^{-1}$, the populations of right and wrong states are $p_r \approx tk_+^r$ and $p_w \approx tk_+^w$. To shorten notation, we define the function $f(x) = e^{-x}/(1 + e^{-x})$ mapping energies into errors. The short-time error is then $\eta(t \rightarrow 0) = f(\delta)$. In the opposite limit of $t \gg \omega^{-1}$, the system reaches equilibrium so that $\eta(t \rightarrow \infty) = f(\gamma)$ by detailed balance. At intermediate times, one can demonstrate from the analytical solution of Eqs. 2 that $\eta(t)$ is a monotonic function for any choice of rates (see [19]): increasing with time when $\delta > \gamma$ (i.e. $f(\delta) < f(\gamma)$), and decreasing when $\gamma > \delta$ (i.e. $f(\delta) > f(\gamma)$). For $\delta = \gamma$, the error is time-independent. Examples of the three cases are shown in Fig. 2b.

To maximize accuracy, the copying reaction must be arrested when $\eta(t)$ is at its minimum value, quenching the system into either a right or wrong copy outcome. In an enzymatic reaction, this corresponds to the irreversible transformation of a bound state into a product [2]. In [8], where bits are encoded in ferromagnets, it corresponds to the decoupling from an external transverse field. It is then natural to define the *kinetic discrimination* regime $\delta > \gamma$, where optimal accuracy requires stopping the process as fast as possible. If $\gamma > \delta$,

energetic discrimination regime, optimal accuracy is reached when the reaction reaches equilibrium, i.e. at very long time. In all cases, accuracy can not be improved by combining the two mechanisms, as the lower limit on the error is determined by either γ or δ . Notice that in an energetic discrimination scheme, the quench can be performed slowly, at no dissipation [8]. In a kinetic scheme, the quench has to be fast and dissipative.

Kinetic and energetic discrimination in copolymerization. In copolymerization, a polymerase continuously adds and removes monomers to a tip of the growing copy strand, trying to match them with the monomers on a template strand (see Fig. 1b). The model is defined by the incorporation and removal rates of right k_{\pm}^r and wrong k_{\pm}^w matching monomers. As before, the rates are given by Eq. 1 and Fig. 2a. The chemical driving for right and wrong bases are thus ϵ and $\epsilon - \gamma$ respectively. Previous studies assumed iso-energetic strands, i.e. $\gamma = 0$ [1, 14, 11, 12]. We relax this assumption and study how the copying velocity v , and the rate of entropy production or dissipated chemical work \dot{S} [20] depend on the error rate η for a general choice of δ and γ . It is straightforward to show that $v = k_{+}^r - (1 - \eta)k_{-}^r + k_{+}^w - \eta k_{-}^w$ [1, 12], and also that \dot{S} is given by

$$\dot{S} = v\Delta S = v(1 - \eta)\epsilon + v\eta(\epsilon - \gamma) + vH(\eta) \quad (3)$$

where $\Delta S = \dot{S}/v$ is the dissipation per step, and $H(\eta) = -\eta \log(\eta) - (1 - \eta) \log(1 - \eta)$ is the Shannon entropy of the error rate η . The first two terms in Eq. (3) represent the distinct chemical driving forces of right and wrong bases, multiplied by the flux of right and wrong incorporated bases. The last term of Eq. (3) corresponds to the information entropy increase due to incorporation of errors, hence information, into the chain [1, 11, 12].

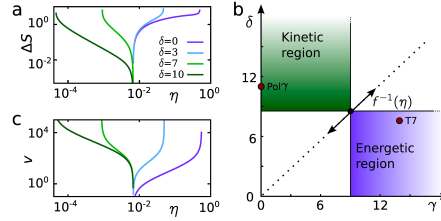


Fig. 3: a) Dissipation per step in copolymerization. In all curves $\gamma = 5$, while δ varies. All curves tend to zero at $\eta = f(\gamma) \approx 6.7 \cdot 10^{-3}$. Blue curves are in the energetic region, $\gamma > \delta$, while green curves are in the kinetic region $\delta > \gamma$. b) γ - δ phase diagram, showing the kinetic and energetic discrimination regions compatible with an error $\eta \sim f(7) \approx 9 \cdot 10^{-4}$, and estimated values of (γ, δ) for Pol γ and T7. Tuning η shifts the limit $f^{-1}(\eta)$ of phase regions along the line $\gamma = \delta$. c) Behavior of the velocity v for the parameter choices in a).

By imposing steady state flux conservation, we obtain the functions $\Delta S(\eta)$ and $v(\eta)$ [19], presented in Fig.3a and 3c for a fixed value of γ and different

values of δ . The range of physically admissible errors η depends on γ and δ (see [19]) as

$$\min[f(\delta), f(\gamma)] < \eta < \max[f(\delta), f(\gamma)], \quad (4)$$

with $f(x)$ as previously defined. We now study the dissipative limit, $\eta \rightarrow f(\delta)$, and the adiabatic limit, $\eta \rightarrow f(\gamma)$.

When $\eta \rightarrow f(\delta)$, the chemical driving diverges as $\epsilon \sim -\log|\eta - f(\delta)|$ [19]. Substituting into Eq. (3) shows that also ΔS diverges (see Fig. 3a) as

$$\Delta S \sim \eta \epsilon \sim \eta \log|\eta - f(\delta)| \quad \text{for } \eta \rightarrow f(\delta). \quad (5)$$

Since $\epsilon \gg 1$, the information entropy $H(\eta)$ in Eq. (3) is negligible, and dissipation is dominated by the chemical terms. As an effect of the strong driving, the velocity diverges as $|\eta - f(\delta)|^{-1}$, see Fig. 3c.

When $\eta \rightarrow f(\gamma)$, both v and ΔS tend to zero, see Fig. 3a and 3c: all the chemical energy is invested in copying the information, none being wasted. The chemical driving is then

$$\epsilon = \log(1 - \eta) = \log[1 - f(\gamma)] < 0 \quad \text{for } \eta = f(\gamma). \quad (6)$$

Note that ϵ is small and *negative*, to compensate the small positive entropic driving caused by $H(\eta)$ in Eq. (3).

By inverting Eq. (4), the values of γ and δ compatible with a given error η must satisfy either $\gamma < f^{-1}(\eta) < \delta$ or $\delta < f^{-1}(\eta) < \gamma$, with $f^{-1}(x) = \log(1 + 1/x)$ the inverse of $f(x)$. This defines the two disconnected *kinetic discrimination* ($\delta > \gamma$), and *energetic discrimination* ($\gamma > \delta$) regions of the (γ, δ) plane in Fig. 3b.

In the kinetic region, both ΔS and v diverge in the minimum error limit, so that accuracy comes at the cost of high dissipation. In the energetic region, accurate copying comes at the cost of the copying velocity, which goes to zero in the adiabatic minimum error limit. This fundamental difference is at the core of the discrepancies between enzymatic copying models [2] that assumed lack of forward discrimination, $\delta = 0$ in our language (see [19] for mapping), and copolymerization studies [1, 11, 12] that assumed iso-energetic strands, $\gamma = 0$. Our results show that it is impossible to interpolate between the two, as they belong to two separate phase space regions.

Operating regimes of T7 and Pol γ polymerases. We now analyze two specific biological copying systems: DNA replication of the phage T7 [2, 21], and replication of human DNA by Pol γ [22]. A recent experimental study [22] points at the strong and asymmetric backward rates as the leading discriminatory mechanism in T7. We derived from [22] the copolymerization rates by assuming equilibrium nucleotide binding with dissociation constants $K_r = 28\mu\text{M}$ and $K_w = 200\mu\text{M}$ for right and wrong base matching. Considering nucleotide concentrations in a range of $[dNTP] \sim 0.5 - 50\mu\text{M}$ we obtain the binding states $1/(1 + K_{r/w}/[dNTP])$. Multiplying them by the forward rates (360Hz and 0.2Hz for right and wrong bases respectively) we obtain $k_+^{r/w}$. The backward rates are $k_-^r \approx 2\text{Hz}$ and $k_-^w \approx 0.04\text{Hz}$ [22]. These values give an error range

$\eta \sim 10^{-6} - 10^{-4}$, in agreement to the estimates in [2]. Usual estimates of the error assume linear binding, approximation valid for low $[dNTP]$ and yielding the lowest end of the error range. The velocities are $v \sim 5 - 250$ bps (bases per second), in agreement with the saturation rate measured in [22]. By inverting Eqs.(1), we can infer $\gamma \approx 14$ and $\delta \approx 8$. Since $\gamma > \delta$, we conclude that T7 operates in the energetic regime (see Fig. 3b).

DNA duplication by Pol γ was analyzed in [11] with a variant of the copolymerization model, where different monomer species are characterised by different rates. Agreement with experimental data in [21] was obtained assuming that the copy be iso-energetic ($\gamma = 0$). We simplify the analysis in [11] by averaging over the different monomer species. Using the same driving $\epsilon \approx 5$ determined for T7, we obtain $\delta \approx 11$ and a range of error rates $\eta \sim 10^{-5} - 10^{-3}$. In the limit of low $[dNTP]$ it agrees with the estimates in [21, 11]. As $\gamma = 0$, Pol γ lies in the kinetic discrimination region (Fig. 3b).

The estimates above indicate that the two polymerases achieve a similar error rate operating in the two different regimes. They thus experience different trade-offs. In T7 lowering $[dNTP]$ (effectively, the chemical driving) reduces the error η and the dissipation ΔS , at the cost of a smaller speed v . This situation is similar to that of the blue curves in Figs. 3a and 3c. In Pol γ smaller error requires a stronger driving, hence dissipation [11]. This gives a higher polymerization rate, as in the green curves of Figs. 3a and 3c.

Combining copying strategies in proofreading schemes. In copolymerization, kinetic and energetic discrimination cannot be combined to reduce the error, Eq. 4. We now explore the possibility of combining the two mechanisms in multi-step copying schemes involving a proofreading pathway. In proofreading, an initially copied base can be removed via an alternative pathway, see Fig. 1c and 1d. Such erasing pathway is characterized by a discrimination which, a priori, can be energetic γ_p or kinetic δ_p , a distinct time-scale $1/\omega_p$, and a (backward) driving ϵ_p . In an effective proofreading scheme, the minimal copying error of Eq. 4 is reduced by an additional proofreading factor, in principle energetic $f(\gamma_p)$ or kinetic $f(\delta_p)$. We discuss two proofreading schemes. In both of them, the proofreading rates $\tilde{k}_{\pm}^{r/w}$ have the same structure as the copying ones, apart from a backward driving [19]. In the first, Fig. 1c, the copying step is identical to that in the copolymerization model, as in Bennett's proofreading model [1]. In the second, Fig. 1d, the copying step leads to an intermediate state, taken to its final form via rates $\bar{k}_{\pm}^{r/w}$ without further discrimination, as in Hopfield's model [2]. By imposing flux balance at the steady state we solved both models analytically [19]. We fixed the discrimination factors, and for each error η minimized ΔS over the remaining free parameters [19], obtaining the curves of minimum dissipation vs. error in Fig. 4.

As shown in [19], there are no regimes in any of the two proofreading schemes where the error is lowered by the energetic factor $f(\gamma_p)$, while error reduction by a kinetic proofreading factor $f(\delta_p)$ is feasible, see Fig. 2c and 2d. Proofreading is thus only effective when it operates in the kinetic regime. This result is consistent with Landauer's principle [23] as, unlike copying, proofreading involves

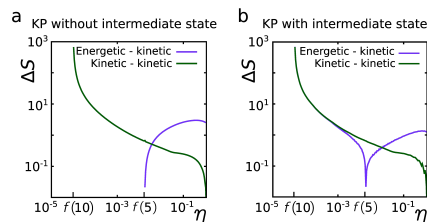


Fig. 4: a) Minimum dissipation in proofreading without an intermediate step, Fig. 1c. For both curves, $\gamma_p = 0$ and $\delta_p = 5$. In the case of energetic discrimination in copying and kinetic discrimination in proofreading (energetic-kinetic), the other parameters are $\gamma = 5$, $\delta = 0$. In the kinetic-kinetic case, we used $\gamma = 0$ and $\delta = 5$. b) Minimum dissipation in proofreading with an intermediate step, Fig. 1d. Parameters are as in a). In both panels, the expected minimum errors $f(10)$ and $f(5)$, depending on whether proofreading is effective or not, are marked.

erasure of information (errors) and thus is intrinsically dissipative. Further, by looking at the minimum errors in Fig. 4, one can conclude that, while kinetic proofreading is always effective when combined with kinetic copying (green curves), it is only compatible with adiabatic copying when an intermediate state is present (blue curves). This is a key difference between the proofreading schemes in [1] and [2]: without an intermediate state it is impossible to find a regime where copies are produced adiabatically, and undone very quickly. The combination of kinetic proofreading with adiabatic copying step has the advantage of a lower dissipation (see Fig. 4b, green vs. blue lines).

In this Letter, we have shown how *each* copying step in stochastic copying can be unambiguously classified into one of two radically different classes, kinetic and energetic discrimination. The existence of an energetic regime in the copolymerization model complements the view in the literature [1, 11, 12] that low copy errors are achieved only in a highly dissipative regime. It also demonstrates how entropy-driven growth, a phenomenon studied in the large error limit [1, 14, 11, 15], can be exploited to reliably copy information. Copolymerization is thus compatible with the principle of reversible computing stating that a copy can be performed adiabatically [8]. The analysis of two DNA polymerases, T7 and Pol γ , shows that the first operates in the energetic regime, while the second in the kinetic one. Both mechanisms are thus used by biological systems. Finally, our study of proofreading proves that the two regimes discussed here are combined in more complex copying schemes.

Our conceptual framework can be applied to a wider range of problems related to stochastic discrimination. Examples are detection of antigens by T-cell receptors [24], and discrimination of a binary input in neural dynamics [25]. At the sub-cellular level, thermal fluctuations dominate and impose constraints on biological tasks. While the thermodynamics of bio-mechanical systems such

as molecular motors is well understood [26], the role of fluctuations in biological information processing such as bacterial chemotaxis presents still many open questions [27]. Our work shows that the emerging trade-offs may be complex, and depend on the region in parameter space where the system operates.

This work was partially supported by a Max Planck Society scholarship (to P.S.) and a Ramon y Cajal Grant (to S.P.). We are grateful to A. Bernacchia, J. Garcia-Ojalvo, N. Mitarai, L. Granger, M. A. Muñoz and Y. Tu for a critical reading of the manuscript.

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1 Supplementary Information

This document contains additional details and derivation of the results presented in the Letter “Energetic vs. kinetic discrimination in biological copying”. The document is organized as follows. Section 1 presents a full solution of the single bit copying model (model A in Fig. 1 of the main text), and a demonstration that the error is always a monotonic function of time. Section 2 details results on the co-polymerization model (model B in the main text). Section 3 illustrates the mapping between Hopfield’s and Bennett’s copying schemes. Finally, section 4 and 5 present details on the proofreading models (models C and D in the main text, respectively).

1.1 Stochastic copying of a single bit

We wish to demonstrate that, for any choice of the rates, the solution of the system of differential equations:

$$\begin{aligned}\dot{r} &= k_+^r(1 - r - w) - k_-^r r \\ \dot{w} &= k_+^w(1 - r - w) - k_-^w w.\end{aligned}\tag{7}$$

with initial condition $r(t = 0) = w(t = 0) = 0$ leads to a time dependent error

$$\eta(t) = \frac{w(t)}{r(t) + w(t)}.\tag{8}$$

being a monotone function of time for all $t > 0$. In particular, $\eta(t)$ either strictly increasing, strictly decreasing or constant depending on the choice of the parameters.

The solution of the system of equations (7) can be obtained with standard methods. First of all, the steady state solution is:

$$\begin{aligned}r_{eq} &= \frac{1}{1 + \frac{k_-^r}{k_+^r} + \frac{k_-^r k_+^w}{k_+^r k_-^w}} \\ w_{eq} &= \frac{1}{1 + \frac{k_-^w}{k_+^w} + \frac{k_-^w k_+^r}{k_+^w k_-^r}}.\end{aligned}\tag{9}$$

Upon defining $\delta r = r - r_{eq}$ and $\delta w = w - w_{eq}$ the time-dependent distances

from the steady state, a lengthy but straightforward calculation leads to

$$\begin{aligned}
\delta r(t) &= \frac{N_- \left\{ -r_{eq} + \frac{w_{eq}}{2k_+^w} [q + \sqrt{q^2 + 4k_+^r k_+^w}] \right\} e^{\lambda_+ t}}{4 + \frac{q^2}{k_+^r k_+^w}} \\
&+ \frac{N_+ \left\{ -r_{eq} + \frac{w_{eq}}{2k_+^w} [q - \sqrt{q^2 + 4k_+^r k_+^w}] \right\} e^{\lambda_- t}}{4 + \frac{q^2}{k_+^r k_+^w}} \\
\delta w(t) &= \frac{-N_- (q + \sqrt{q^2 + 4k_+^r k_+^w}) \left\{ -r_{eq} + \frac{w_{eq}}{2k_+^w} [q + \sqrt{q^2 + 4k_+^r k_+^w}] \right\} e^{\lambda_+ t}}{2k_+^r \left(4 + \frac{q^2}{k_+^r k_+^w} \right)} \\
&- \frac{N_+ (q - \sqrt{q^2 + 4k_+^r k_+^w}) \left\{ -r_{eq} + \frac{w_{eq}}{2k_+^w} [q - \sqrt{q^2 + 4k_+^r k_+^w}] \right\} e^{\lambda_- t}}{2k_+^r \left(4 + \frac{q^2}{k_+^r k_+^w} \right)}
\end{aligned} \tag{10}$$

where we defined the eigenvalues

$$\lambda_{\pm} = \frac{-\Sigma \pm \sqrt{\Sigma^2 - 4c}}{2} \tag{11}$$

with $\Sigma = k_+^r + k_-^r + k_+^w + k_-^w$ and $c = (k_+^r + k_-^r)(k_+^w + k_-^w) - k_+^r k_+^w$, and the quantities

$$\begin{aligned}
q &= (k_+^r + k_-^r - k_+^w - k_-^w) \\
\mathcal{N}_{\pm} &= 2 + \frac{q}{2k_+^r k_+^w} \left(q \pm \sqrt{q^2 + 4k_+^r k_+^w} \right).
\end{aligned} \tag{12}$$

We now study the sign of the derivative of the error $\eta(t)$. Clearly, its sign is the same as the sign of the derivative of the function

$$f(t) = \frac{w(t)}{r(t)} = \frac{w_{eq} + \delta w(t)}{r_{eq} + \delta r(t)}. \tag{13}$$

The derivative of $f(t)$, before any simplification, reads:

$$\begin{aligned}
f' &= D^{-2} \left[2k_+^w (k_-^w - k_-^r) (k_-^w k_+^r + k_-^r (k_-^w + k_+^w)) e^{-(3\Sigma + \sqrt{Q})t/2} \right] \\
&\left\{ -2Q^2 e^{(\Sigma + \sqrt{Q})t/2} + e^{\Sigma t} [k_-^{r2} + k_-^{w2} + k_-^r (-2k_-^w + 2k_+^r - 2k_+^w + \sqrt{Q}) \right. \\
&+ (k_+^r + k_+^w)(k_+^r + k_+^w + \sqrt{Q}) + k_-^w (-2k_+^r + 2k_+^w + \sqrt{Q})] \\
&+ e^{(\Sigma + \sqrt{Q})t} [k_-^{r2} + k_-^{w2} - k_-^w (-2k_+^w + 2k_+^r + \sqrt{Q}) \\
&\left. - (k_+^r + k_+^w)(-k_+^r - k_+^w + \sqrt{Q}) + k_-^r (-2k_+^r + 2k_-^w + 2k_+^w + \sqrt{Q})] \right\} \tag{14}
\end{aligned}$$

where Σ and q are defined above, and we have also defined the function of the rates $Q = q^2 + 4k_+^r k_+^w$. The denominator D has a complicated expression that

we omit since it is squared, hence it does not contribute to the sign. In the nominator, the term in square brackets clearly has the sign of $k_-^w - k_-^r$. We now move to the study the sign of the term in curly brackets, which can be expressed in terms of hyperbolic functions as

$$\{\} = e^{\frac{1}{2}(\Sigma + \sqrt{Q})t} \left(-2Q + 2e^{\Sigma \frac{t}{2}} \left[Q \cosh(\sqrt{Q}t/2) - \sqrt{Q}\Sigma \sinh(\sqrt{Q}t/2) \right] \right) \quad (15)$$

The prefactor is positive, and we are left with the two terms inside the parenthesis. The first is clearly negative. To determine the sign of the second, particularly the term in brackets, one should note that $\sinh(x) > \cosh(x)$ for all positive x , and that $\Sigma > \sqrt{Q}$, which is shown by expanding the squares. As a consequence, the second term in the brackets is always larger than the first, so that the whole term inside the parenthesis is negative. It follows that the term in the curly brackets is negative, and that the sign of f' is just the sign of $k_-^r - k_-^w$. From this we conclude that the error grows monotonically for all parameter choices such that $k_-^r - k_-^w$.

Finally, the rates are parametrized in the main text by their kinetic and energetic discriminations (δ and γ), the driving ϵ , and an overall time-scale ω . The kinetic discrimination δ appears in the forward rates, so that $k_+^r/k_+^w = e^\delta$. The driving ϵ is defined for right bases, so that $k_+^r/k_-^r = e^\epsilon$. Finally, the energetic discrimination γ reduces the driving of wrong bases, $k_+^w/k_-^w = e^{\epsilon-\gamma}$. Summarizing, we have:

$$k_+^r = \omega e^{\epsilon+\delta} ; \quad k_-^r = \omega e^\delta ; \quad k_+^w = \omega e^\epsilon ; \quad k_-^w = \omega e^\gamma. \quad (16)$$

The condition $k_-^r - k_-^w$ is then equivalent to $\delta > \gamma$, which we termed the case of *kinetic discrimination*. Conversely, when $k_-^r < k_-^w$ (equivalent to $\delta < \gamma$) the error decreases monotonically (regime of *energetic discrimination*).

1.2 Co-polymerization model

Given the four rates k_+^w , k_-^w , k_+^r , and k_-^r , one can easily write the two rate equations as:

$$\begin{aligned} \partial_t[\&r] &= [\&]k_+^r - [\&r]k_r^- \\ \partial_t[\&w] &= [\&]k_+^w - [\&w]k_w^- \end{aligned} \quad (17)$$

Following Bennett's original approach [1], we consider the steady state in which there are constant fluxes of wrong $\partial_t[\&w] = J_w = \eta v[\&]$ and right $\partial_t[\&r] = J_r = (1 - \eta)v[\&]$ additions of aminoacids into the copied strain. Under these assumptions, one can show [1, 3] that the error is given by:

$$\frac{k_+^w - k_-^w \eta}{k_+^r - k_-^r (1 - \eta)} = \frac{\eta}{1 - \eta}. \quad (18)$$

while the average copying velocity is

$$v = k_+^w - k_-^w \eta + k_+^r - k_-^r (1 - \eta). \quad (19)$$

Using the same parametrization of the previous model, Eq. (16), we write the chemical driving ϵ as a function of the energetic discrimination energy γ , the kinetic discrimination energy δ , and the steady state error η . The expression reads

$$\epsilon = \log \left[\eta(1 - \eta) \frac{1 - e^{\gamma - \delta}}{\eta - (1 - \eta)e^{-\delta}} \right]. \quad (20)$$

By means of (20), the average velocity can be expressed as

$$v = \omega \frac{1 - (1 + e^\gamma)\eta}{\eta - (1 - \eta)e^{-\delta}}. \quad (21)$$

We now want to impose that 1) the argument of the logarithm in (20) has to be positive, and 2) the average velocity (21) should also be positive. Assuming of course $0 < \eta < 1$, the first condition is equivalent to $(\delta - \gamma)[\eta - (1 + e^\delta)^{-1}] > 0$, while the second is equivalent to $[(1 - e^\gamma)^{-1} - \eta][\eta - (1 + e^\delta)^{-1}] > 0$. Combining these two conditions leads to Eq. (4) in the main text.

Finally, the dissipation per step is

$$\begin{aligned} \Delta S &= \eta \log \left[\frac{1}{\eta} \right] + (1 - \eta) \log \left[\frac{1}{1 - \eta} \right] + \eta \log \left[\frac{k_+^w}{k_-^w} \right] + (1 - \eta) \log \left[\frac{k_+^r}{k_-^r} \right] \\ &= \left(\eta \log \left[\frac{1}{\eta} \right] + (1 - \eta) \log \left[\frac{1}{1 - \eta} \right] \right) + (1 - \eta)\epsilon + \eta(\epsilon - \gamma), \end{aligned} \quad (22)$$

which can be expressed as a function of η , δ and γ only by using Eq. (20).

1.3 Mapping of Hopfield's original model

In Hopfield's formulation [2], given the template c , by interacting through C and D , either the aminoacid P_C or P_D can be added to an RNA chain. Addition of P_C will be the right addition, and addition of P_D will be considered an error. The rate equation and steady state solution are:

$$c + C \xrightleftharpoons[k_C]{k'_C} [cC] \xrightarrow{v} P_C \quad \text{and} \quad v[Cc] = k'_C[C] - k_C[Cc] \quad (23)$$

and analogously for D . It is assumed that $[C] \sim [D]$, and defined $f_C = [Cc]/[C]$ and $f_D = [Dc]/[D]$ as the fraction of incorporated C and D monomers given a template c . At steady state $f_C = 1 - f_D$, and f_D is the error $\eta = f_D$. Solving the system above we arrive at

$$\frac{\eta}{1 - \eta} = \frac{f_D}{f_C} = \frac{k'_D v + k_C}{k'_C v + k_D} \quad (24)$$

Identifying these rates with those in our model according to Fig. 1, the mapping to Hopfield's model is finished: $k_C = k_-^r$, $k'_C = k_+^r$, $k_D = k_-^w$ and $k'_D = k_+^w$.

To verify the mapping we study two limiting cases. For $\gamma = 0$ (as Bennett assumed in [1]) we have that if $v \rightarrow \infty$, then $\eta/(1 - \eta) \rightarrow k'_D/k'_C = e^{-\delta}$; and

if $v \rightarrow 0$, then $\eta/(1 - \eta) \rightarrow k'_D k_C / k'_C k_D = 1$. On the other hand for $\delta = 0$ (as Hopfield assumed in [2]) we have that if $v \rightarrow \infty$, then $\eta/(1 - \eta) \rightarrow k'_D / k'_C = 1$; and if $v \rightarrow 0$, the classical result is obtained $\eta/(1 - \eta) \rightarrow k'_D k_C / k'_C k_D = k_C / k_D = e^{-\gamma}$, in exact agreement with the results obtained above.

1.4 Proofreading model without intermediate state

A minimal model of Kinetic Proofreading (KP) requires at least two different pathways. The first is the copying pathway introduced above, characterized by a driving which tends to make the chain grow. On the other hand, the driving of the second pathway is *backward*, thus undoing copies on average. The copying pathway has a bias towards adding right bases by a faster (kinetic) and more stable (energetic) binding. Conversely, the proofreading pathway has a bias towards removing wrong bases by a faster and less stable unbinding. The combination of both can reduce the minimal error given by the standard copy, by the discrimination factor of the proofreading pathway. The simplest proofreading scheme consists of the copying scheme introduced before, and a parallel reaction which we characterize by four additional proofreading rates $\tilde{k}_{\pm}^{r/w}$.

1.4.1 Rates parametrization

We choose the same copying rates of the standard copying scheme, see Eq.(16). Further, we introduce proofreading rates which are analogously characterized by a kinetic and energetic proofreading discrimination factors (δ_p and γ_p), a backward driving ϵ_p , and an additional time-scale ω_p . In the case of proofreading, we define the driving in the backward right additions, that is $\tilde{k}_-^r / \tilde{k}_+^r = e^{\epsilon_p}$. The kinetic discrimination is also backwards, and so $\tilde{k}_-^w / \tilde{k}_+^w = e^{\delta_p}$. Finally, the energetic discrimination is reflected in a higher backward driving of wrong bases, such that $\tilde{k}_-^w / \tilde{k}_+^w = e^{\epsilon_p + \gamma_p}$. One can then write the proofreading rates as

$$\tilde{k}_-^r = \omega_p e^{\epsilon_p - \delta_p} ; \quad \tilde{k}_+^r = \omega_p e^{-\delta_p} ; \quad \tilde{k}_-^w = \omega_p e^{\epsilon_p} ; \quad \tilde{k}_+^w = \omega_p e^{-\gamma_p}. \quad (25)$$

The energy levels corresponding to this parametrization of the rates are illustrated in Fig. (5). Notice that the end-states in the proofreading pathway have a difference in energy $\gamma - \gamma_p$. While in some coarse grained models such a behaviour may be justifiable through external agents, typically one would expect this difference not to exist, so that in the main text we always fixed $\gamma_p = \gamma$. Further, we anticipate that numerical results show that the proofreading step is always kinetic. This means that the value of γ_p , as soon as it is positive, will not anyway affect the minimum error achievable by the system.

KP with no intermediate state

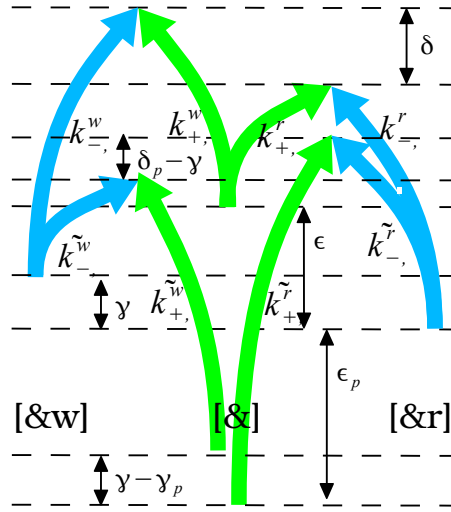


Fig. 5: Energy diagram of the reactions corresponding to the proofreading scheme with no intermediate steps.

1.4.2 Solving the model

The kinetic equations in this case are:

$$\begin{aligned}\partial_t[\&r] &= [\&](k_{+}^r + \tilde{k}_{+}^r) - [\&r](k_{-}^r + \tilde{k}_{-}^r) \\ \partial_t[\&w] &= [\&](k_{+}^w + \tilde{k}_{+}^w) - [\&w](k_{-}^w + \tilde{k}_{-}^w).\end{aligned}\quad (26)$$

Also in this case, the steady state solution can be obtained by considering the fluxes of right and wrong bases added: $\partial_t[\&w] = J_w = \eta v[\&]$ and right $\partial_t[\&r] = J_r = (1 - \eta)v[\&]$. The error as a function of the rates is analogous to the one for simple copying:

$$\frac{k_{+}^w + \tilde{k}_{+}^w - \eta(k_{-}^w + \tilde{k}_{-}^w)}{k_{+}^r + \tilde{k}_{+}^r - (1 - \eta)(k_{-}^r + \tilde{k}_{-}^r)} = \frac{\eta}{1 - \eta}. \quad (27)$$

The next step is to derive from this expression the driving ϵ as a function of the error, the discriminations, and the two new additional parameters: the

proofreading driving ϵ_p and its characteristic time scale ω_p . The result is:

$$\epsilon = \log \left[\frac{1}{1 - \eta(1 + e^\delta)} \left\{ \eta(1 - \eta)(e^\gamma - e^\delta + \omega_p e^{\epsilon_p} - \omega_p e^{\epsilon_p - \delta_p}) - \omega_p e^{-\gamma_p} + \eta \omega_p (e^{-\gamma_p} + e^{-\delta_p}) \right\} \right] \quad (28)$$

The velocity is also analogous to that of the simple copying scheme:

$$v = k_+^w + \tilde{k}_+^w - \eta(k_-^w + \tilde{k}_-^w) + k_+^r + \tilde{k}_+^r - (1 - \eta)(k_-^r + \tilde{k}_-^r). \quad (29)$$

However, for the entropy production rate, one has to consider the transitions corresponding to the two pathways independently:

$$\begin{aligned} \dot{S} = & (k_+^w - \eta k_-^w) \log \left[\frac{k_+^w}{\eta k_-^w} \right] + (k_+^r - (1 - \eta)k_-^r) \log \left[\frac{k_+^r}{(1 - \eta)k_-^r} \right] \\ & + (\tilde{k}_+^w - \eta \tilde{k}_-^w) \log \left[\frac{\tilde{k}_+^w}{\eta \tilde{k}_-^w} \right] + (\tilde{k}_+^r - (1 - \eta)\tilde{k}_-^r) \log \left[\frac{\tilde{k}_+^r}{(1 - \eta)\tilde{k}_-^r} \right]. \quad (30) \end{aligned}$$

Finally, the dissipation per step is simply calculated as $\Delta S = \dot{S}/v$.

1.4.3 Minimization procedure and numerical results

For each given value of the error η and the four parameters γ , γ_p , δ , δ_p , we identified the values of the two remaining free parameters ω_p and ϵ_p corresponding to the minimum dissipation per step. In order to avoid local minima, we adopted a systematic minimization scheme: the two parameters have been varied with a logarithmic step equal to 1.04, in an interval $10^{-5} < \omega_p, \epsilon_p < 10^9$. In this region, we found the minimum dissipation per step with the constraint of a positive reaction velocity. We also checked a posteriori that no minimum was found at the boundaries of the minimization region.

A systematic simulation study of the 4 possibilities of energetic/kinetic copy coupled to energetic/kinetic proofreading is presented in Fig. (6). The results allows us for reaching the following conclusions:

- The proofreading pathway can reduce the minimum error in the kinetic regime only. This can be seen in the lower panels of Fig. (6), where increasing γ_p does not affect the minimum achievable error. In particular, in the bottom left panel the copying is energetic and the minimum error is given by $f(\gamma) = e^{-\gamma}/(1 + e^{-\gamma}) \approx 0.0067$ for $\gamma = 5$. In the bottom right panel, the copying is kinetic and again the minimum error is given by $f(\delta) \approx 0.0067$ for $\delta = 5$. The minima in the two figures correspond to parameters such as the proofreading reactions has an average forward flux instead of backward, so that the proofreading pathway works as an effective parallel adiabatic (energetic) copying pathway.
- cooperative error reduction only takes place when both pathways are in the kinetic region. In the top right panel, increasing δ_p does not reduce the

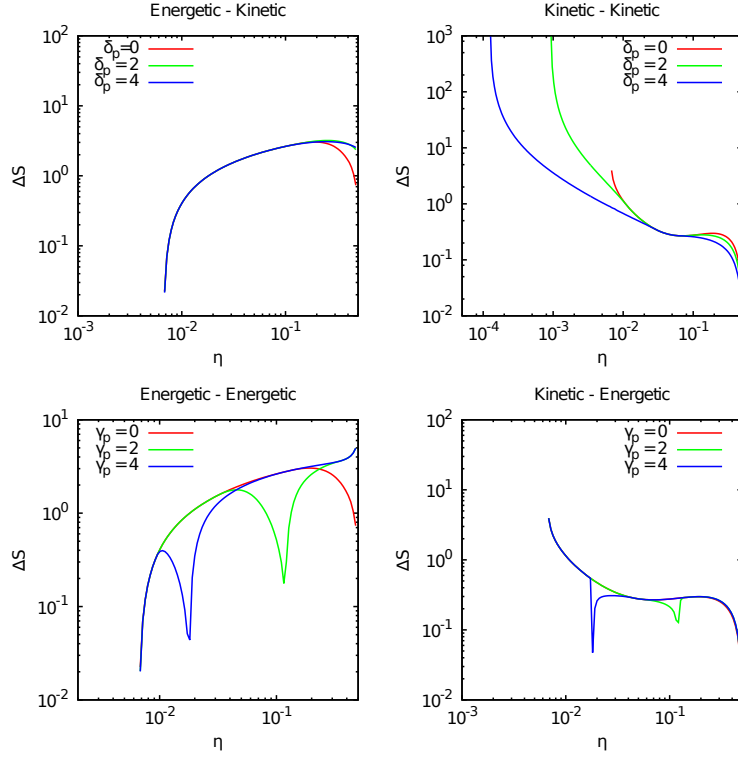


Fig. 6: Study of the four possible combination of energetic/kinetic discrimination and energetic/kinetic proofreading in Bennett's model. In the left panels the copy is energetic; in particular we chose $\delta = 0$ and $\gamma = 5$. Conversely, in the right panels the copy is kinetic with $\delta = 5$ and $\gamma = 0$. In the top panels the proofreading scheme is purely kinetic ($\gamma_p = 0$), while in the bottom panel we fixed $\delta_p = 0$ and varied γ_p .

error. The only case in which the error can be reduced is in the kinetic-kinetic case of the top right panel, where the minimum error is given by $f(\delta)f(\delta_p) \approx f(\delta + \delta_p) \approx 0.0067, 0.0009, 0.0001$ for $\delta_p = 0, 2, 4$ respectively. We remark that this feature is a peculiarity of this model. We will show in the next section how including an intermediate state in the copying pathway allows for error reduction with an energetic copy and a kinetic proofreading.

1.5 Proofreading model with intermediate state

In this section we present more extensive results on model 4 of the main text. This model presents some analogies with the previous one, except that copying

occurs via an intermediate state, denoted with a “*”, which is connected with the final state in which the aminoacid is incorporated. This final state has also a proofreading step. The overall reaction scheme is more in the spirit of Hopfield’s original proofreading mechanism.

1.5.1 Parametrization of the rates

The forward copying rates from the unbound to the intermediate state are defined in exactly the same way as the copying rates in the previous models, see Eq. (16). As in Hopfield’s original model, the transition rates from the intermediate state to the final state have no discrimination, but have their own driving ϵ^* and time scale given by ω^* . They obey the relations $\bar{k}_+^w/\bar{k}_+^r = 1$, $\bar{k}_+^w/\bar{k}_-^w = e^{\epsilon^*}$ and $\bar{k}_+^r/\bar{k}_-^r = e^{\epsilon^*}$. It is not hard to show that adding a discrimination below that of the original copying does not reduce the error beyond the critical error. Adding a bigger one simply reduces it to the critical error of this secondary copy, unlike the additive effect of proofreading. The rates can be simply written as:

$$\bar{k}_+^r = \omega^* e^{\epsilon^*} ; \bar{k}_-^r = \omega^* ; \bar{k}_+^w = \omega^* e^{\epsilon^*} ; \bar{k}_-^w = \omega^* \quad (31)$$

The final state is then connected with the initial state by the same proofreading rates defined in the previous section, Eq. (25). The full energy diagram is depicted in Fig. (7). As before, the energy difference $\gamma - \gamma_p$ is irrelevant as the proofreading step has to be a kinetic step, and so we choose it arbitrarily to be null. Again, this corresponds to the physical requirement that the energy of the chain can not change if no base is added.

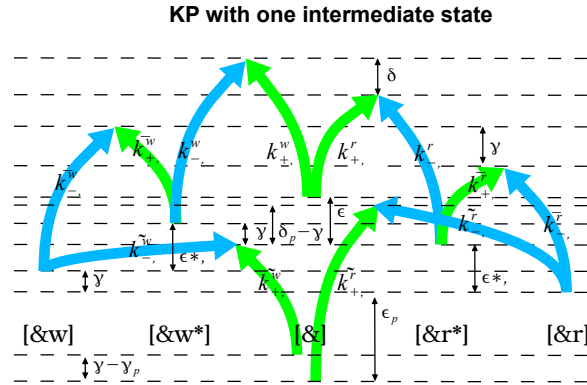


Fig. 7: Energy diagram of the reactions corresponding to the proofreading scheme with an intermediate step.

1.5.2 Solving the model

With the notation introduced in the previous section, it is easy to write the four kinetic equations of this proofreading scheme:

$$\begin{aligned}
\partial_t[R] &= [R^*]\bar{k}_+^r + [\&]\tilde{k}_+^r - [R](\bar{k}_-^r + \tilde{k}_-^r) \\
\partial_t[W] &= [W^*]\bar{k}_+^w + [\&]\tilde{k}_+^w - [W](\bar{k}_-^w + \tilde{k}_-^w) \\
\partial_t[R^*] &= [\&]k_+^r + [R]\bar{k}_-^r - [R^*](k_-^r + \bar{k}_+^r) \\
\partial_t[W^*] &= [\&]k_-^w + [W]\bar{k}_+^w - [W^*](k_-^w + \bar{k}_+^w).
\end{aligned} \tag{32}$$

The easiest way to obtain the solution is by flux balance at the steady state of constant growth velocity v , which corresponds to:

$$\begin{aligned}
[W]v &= ([\&]\tilde{k}_+^w - [W]\tilde{k}_-^w) + ([W^*]\bar{k}_+^w - [W]\bar{k}_-^w) \\
[\&]k_+^w - [W^*]k_-^w &= [W^*]\bar{k}_+^w - [W]\bar{k}_-^w \\
[R]v &= ([\&]\tilde{k}_+^r - [R]\tilde{k}_-^r) + ([R^*]\bar{k}_+^r - [R]\bar{k}_-^r) \\
[\&]k_+^r - [R^*]k_-^r &= [R^*]\bar{k}_+^r - [R]\bar{k}_-^r.
\end{aligned} \tag{33}$$

As before, we seek equations to determine the error rate and the velocity as a function of the rates. We proceed by dividing each of the equations in section by $\&$ and define $W/\& = \eta$, $R/\& = (1 - \eta)$, $W^*/\& = w^*$, $R^*/\& = r^*$. By means of the 2nd and 4th equations we find an expression for r^* and w^* :

$$\begin{aligned}
w^* &= \frac{k_+^w + \eta\bar{k}_-^w}{k_-^w + \bar{k}_+^w} \\
r^* &= \frac{k_+^r + (1 - \eta)\bar{k}_-^r}{k_-^r + \bar{k}_+^r}.
\end{aligned} \tag{34}$$

Substituting into the other 2 equations lead to two coupled equations for η and v .

$$\begin{aligned}
\eta v &= (\tilde{k}_+^w - \eta\tilde{k}_-^w) + \left[\bar{k}_+^w \frac{k_+^w + \eta\bar{k}_-^w}{k_-^w + \bar{k}_+^w} - \eta\bar{k}_-^w \right] \\
(1 - \eta)v &= [\bar{k}_+^r - (1 - \eta)\bar{k}_-^r] + \left[\bar{k}_+^r \frac{k_+^r + (1 - \eta)\bar{k}_-^r}{k_-^r + \bar{k}_+^r} - (1 - \eta)\bar{k}_-^r \right].
\end{aligned} \tag{35}$$

Now we multiply the first equation by $(1 - \eta)$, the second by η and subtract the second from the first to find a closed expression for η :

$$\begin{aligned}
(1 - \eta)(\tilde{k}_+^w - \eta\tilde{k}_-^w) + (1 - \eta) \left[\bar{k}_+^w \frac{k_+^w + \eta\bar{k}_-^w}{k_-^w + \bar{k}_+^w} - \eta\bar{k}_-^w \right] \\
- \eta[\bar{k}_+^r - (1 - \eta)\bar{k}_-^r] - \eta \left[\bar{k}_+^r \frac{k_+^r + (1 - \eta)\bar{k}_-^r}{k_-^r + \bar{k}_+^r} - (1 - \eta)\bar{k}_-^r \right] = 0.
\end{aligned} \tag{36}$$

Again, this formula can be inverted to obtain the copying driving ϵ as a function of η and the other energy differences:

$$e^\epsilon = \frac{\eta\omega_p e^{-\delta_p} - (1-\eta)\omega_p e^{-\gamma_p} + \eta(1-\eta) \left(\omega_p e^{\epsilon_p} (1 - e^{-\delta_p}) + \frac{(\omega^{*2} e^{\epsilon^*})(e^\gamma - e^\delta)}{(e^\delta + \omega^* e^{\epsilon^*})(e^\gamma + \omega^* e^{\epsilon^*})} \right)}{\omega^* e^{\epsilon^*} \left(\frac{1-\eta}{e^\gamma + \omega^* e^{\epsilon^*}} - \frac{\eta e^\delta}{e^\delta + \omega^* e^{\epsilon^*}} \right)}. \quad (37)$$

The velocity is straightforward to calculate from one of the expressions in (35), and is simply:

$$v = \left(\frac{\tilde{k}_+^w}{\eta} - \tilde{k}_-^w \right) + \left[\frac{\bar{k}_+^w k_+^w + \eta \bar{k}_-^w}{\eta k_-^w + \bar{k}_+^w} - \bar{k}_-^w \right] \quad (38)$$

Finally, we calculate the entropy production by summing the six contributions of the local fluxes of the system. This results in the following lengthy expression:

$$\begin{aligned} \&\dot{S} &= (\&k_+^w - W^* k_-^w) \log \left[\frac{\&k_+^w}{W^* k_-^w} \right] + (W^* \bar{k}_+^w - W \bar{k}_-^w) \log \left[\frac{W^* \bar{k}_+^w}{W \bar{k}_-^w} \right] \\ &+ (\&\tilde{k}_+^w - W \tilde{k}_-^w) \log \left[\frac{\&\tilde{k}_+^w}{W \tilde{k}_-^w} \right] + (\&k_+^r - R^* k_-^r) \log \left[\frac{\&k_+^r}{R^* k_-^r} \right] \\ &+ (R^* \bar{k}_+^r - R \bar{k}_-^r) \log \left[\frac{R^* \bar{k}_+^r}{R \bar{k}_-^r} \right] + (\&\tilde{k}_+^r - R \tilde{k}_-^r) \log \left[\frac{\&\tilde{k}_+^r}{R \tilde{k}_-^r} \right]. \end{aligned} \quad (39)$$

Dividing by $\&$ and using the expressions for r^* , w^* and η , we obtain the rate of entropy production:

$$\begin{aligned} \dot{S} &= \left(k_+^w - \frac{k_+^w + \eta \bar{k}_-^w}{k_-^w + \bar{k}_+^w} k_-^w \right) \log \left[\frac{(k_-^w + \bar{k}_+^w) k_+^w}{(k_+^w + \eta \bar{k}_-^w) k_-^w} \right] \\ &+ \left(\frac{k_+^w + \eta \bar{k}_-^w}{k_-^w + \bar{k}_+^w} \bar{k}_+^w - \eta \bar{k}_-^w \right) \log \left[\frac{(k_+^w + \eta \bar{k}_-^w) \bar{k}_+^w}{(k_-^w + \bar{k}_+^w) \eta \bar{k}_-^w} \right] \\ &+ (\tilde{k}_+^w - \eta \tilde{k}_-^w) \log \left[\frac{\tilde{k}_+^w}{\eta \tilde{k}_-^w} \right] \\ &+ \left(k_+^r - \frac{k_+^r + (1-\eta) \bar{k}_-^r}{k_-^r + \bar{k}_+^r} k_-^r \right) \log \left[\frac{(k_-^r + \bar{k}_+^r) k_+^r}{(k_+^r + (1-\eta) \bar{k}_-^r) k_-^r} \right] \\ &+ \left(\frac{k_+^r + (1-\eta) \bar{k}_-^r}{k_-^r + \bar{k}_+^r} \bar{k}_+^r - (1-\eta) \bar{k}_-^r \right) \log \left[\frac{(k_+^r + (1-\eta) \bar{k}_-^r) \bar{k}_+^r}{(k_-^r + \bar{k}_+^r) (1-\eta) \bar{k}_-^r} \right] \\ &+ (\tilde{k}_+^r - (1-\eta) \tilde{k}_-^r) \log \left[\frac{\tilde{k}_+^r}{(1-\eta) \tilde{k}_-^r} \right]. \end{aligned} \quad (40)$$

1.5.3 Minimization procedure and numerical results

In analogy with the previous model, for each value of the parameters δ , δ_p , γ and γ_p and the variable η we found the values of the free parameters corresponding to

the minimum dissipation per step. In this case we had to minimize with respect to four free parameters: ω_p , ϵ_p , ω^* and ϵ^* . Given the number of parameters, we implemented a larger logarithmic minimization step, equal to 1.2.

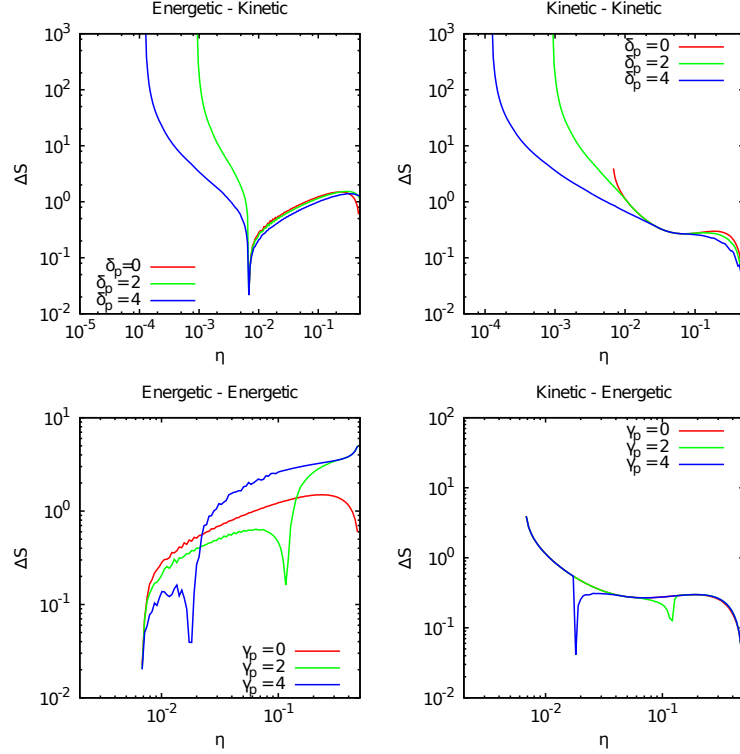


Fig. 8: Study of the four possible combination in the proofreading model with an intermediate state. In the left panels the copy is energetic; in particular we chose $\delta = 0$ and $\gamma = 5$. Conversely, in the right panels the copy is kinetic with $\delta = 5$ and $\gamma = 0$. In the top panels the proofreading scheme is purely kinetic ($\gamma_p = 0$), while in the bottom panel we fixed $\delta_p = 0$ and varied γ_p .

The result of Fig. (8) are consistent to those of the previous model, see Fig. (6). The only important difference is:

- The presence of an additional step in the copying pathway allows for error reduction via an energetic copy - kinetic proofreading scheme. This can be seen in the top left panel of Fig. (8), where the minimum error does depend on δ_p via the usual function $f(\gamma)f(\delta_p) \approx f(\gamma + \delta_p)$. This is at variance with model 3, shown in Fig. (6), where the minimum error in the same case was simply equal to $f(\gamma)$.

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